Supplementary Materials:

Materials and Methods

Figures S1-S8

Table S1-3

References (1-13)

Materials and Methods:

Animals. Adult male wistar rats were used for all experiments. Animals were individually housed in polypropylene cages (57 x 35 x 20 cm) with abundant pine bedding in a temperature- (23C) and light- (0700-1900 h) controlled room. All animals had *ad libitum* access to standard food and water. Upon arrival to the facility, animals were allowed to habituate to the vivarium for one week and were then handled for 2 min/d during 3 days prior to the start of all experiments. All behavioral manipulations were performed during the light phase by experimenters blind to treatment groups. Unless otherwise indicated, all animals were first characterized for trait anxiety and then sacrificed under basal conditions for ex-vivo analyses. All efforts were made to minimize the number of animals while maintaining statistical rigor.

Statistical analyses. All samples represent biological replicates. Sample sizes are indicated in the figure legends. Unpaired two-tailed Student's t-tests were used to compare sets of data obtained from independent groups of animals. In follow-up experiments, unpaired one-tailed Student's t-tests were used to confirm previous findings. Within-pair amounts of behavior in the social competition test were compared using paired two-tailed Student's t-tests. In sets of data in which animals were matched for anxiety, relative social dominance scores were compared using one-sample t-tests against chance (50%). When necessary, data were transformed to obtain symmetric distributions for statistical analysis. Figures are presented with original, non-transformed data. All data –except for mitochondrial respiration- were analyzed using Prism version 5.01 (Graphpad software Inc., San Diego, CA). For respiration experiments, data were analyzed using SPSS statistical software version 13.0 (SPSS, Chicago, IL). As experiments were performed in blocks across days, a linear mixed model was created that included block as a random effect in addition to fixed effects of either anxiety or treatment (where appropriate). The estimated marginal means of the model are then reported. P-values are reported in figure legends, with the second decimal rounded to the nearest figure. Statistical significance was considered at the $p<0.05$ level.

Elevated Plus Maze. Prior to the testing for social hierarchy, animals were tested for anxiety-related behavior in the EPM as previously described (1). Lighting was maintained at 15-16 lx on the open arms and 5-7 lx in the closed. Depending on the amount of time spent on the open arm animals were classified as high- (HA, ≤5% open arm duration) intermediate (IA, 6-20 % open arm duration) or low-anxious (LA, ≥20% open arm duration). Before and in between testing the apparatus was cleaned with a 5 % EtOH solution.

Light-Dark box. To ascertain that the anxiety-like behavior measured in the EPM is indeed a reliable indicator for anxiety; animals were also tested in the light-dark box, 2 d after EPM-exposure. The apparatus and procedure are the same as that described in a previous publication (1), with the modification of lighting intensity. One chamber was black (dark box) and kept at 20lx while the other chamber was white (light box) with an illumination level of 160lx. Before and in between testing the apparatus was cleaned with a 5% EtOH solution.

Social hierarchy test. Rats were pair-wise matched for weight but, depending on the purpose of the experiment, animals in each pair were either of a similar or opposite anxiety profile. In experiments that aimed at disentangling the impact of anxiety in the formation of a social hierarchy, rat pairs were composed of a high- and a low-anxious rat. In pharmacological experiments addressed to investigate the impact of specific treatments on social dominance, pairs of animals were matched for similar anxiety levels; i.e., the rats in each dyad were considered equal in their probability (= 50%) to become dominant or subordinate during their encounter.

As previously described (2), animals were marked on their body for identification and placed in pairs in a clean (neutral) cage without food or water for 20 min. During the social hierarchy test both rats displayed spontaneously offensive behavior, but this balance typically shifted in the favor of one animal towards the end of the test. Social dominance was estimated by summation of the total duration of offensive behaviors for each rat in the dyad (offensive upright, lateral threat and keeping-down behavior, as previously described (3)). Earlier we found that social dominance in these encounters correlate with the outcome in competition for water or for food rewards (1). During pilot studies, we found that infrequently offensive behavior was virtually absent during social encounter (no rat displaying >10s of total offensive behavior); these pairs were excluded from analysis as the relative social dominance in these pairs cannot be reliably measured. Auto-grooming and social investigation was taken into account to determine the specificity of the drug-effects on offensive behavior.

Social preference test. The social preference test was performed in a rectangular, three-chambered box that included a central compartment where the rat was initially placed. Thereafter, retractable doors were removed and the rat could explore the left- and right-compartment for 5 min. The left- and right-compartments were equipped with a floor-fixed transparent perforated Plexiglas cylinder that contained either an unfamiliar male juvenile rat or an unfamiliar object. The time spent sniffing either the juvenile (social target) or the novel object (inanimate target) was manually scored from videotapes by an experimenter who was blinded to the treatment groups.

Open Field test. The open field was used to determine the effects of intra-NAc and intra-BLA infusion of the mitochondrial complex I-and II inhibitors rotenone, 3-nitroproprionic acid, and malonic acid on locomotor behavior. The open field apparatus and procedure were previously described (1). The light was adjusted to a level of 8-10 lx in the center of the arena.

In situ hybridization. Rats were characterized for anxiety and sacrificed either under basal conditions or 5 min after a paired social competitive encounter. Brains were rapidly dissected out, frozen at -30°C in isopentane and stored at -80°C until further processing. 20μm sections were obtained on a freezing cryostat and prepared and hybridized as previously described (4). An outline was created for each region of interest from the left and right sides of the brain from rostral/caudal sections. The radioactive signal was quantified from 8-12 brain sections per region per rat using MCID Image Analysis software (MCID, UK). Optical density values were background-corrected, multiplied by the area sampled to produce an integrated density measurement, and then averaged to produce one data point for each brain region for each animal for statistical analysis.

Corticosterone Analysis. Rats were characterized for anxiety and sacrificed either in basal conditions or 5 min after a paired social competitive encounter. Trunk blood was collected and centrifuged at 12,000 rpm to isolate plasma. 10μL of plasma sample were then prepared according to manufacturer's instructions to measure corticosterone concentrations using a corticosterone ELISA kit (Enzo Life Sciences, ADI-901-097). Levels were calculated using a standard curve method.

Intracerebral cannulation surgery. Rats subjected to pharmacological experiments were implanted bilateral with stainless steel guide cannulas aimed at the NAc or BLA. Rats were anesthetized by isoflurane inhalation (induction 4% isoflurane for 4 min and maintenance 2.5% isoflurane in O_2 at a flow of 4L/min) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Small holes were drilled through the skull for bilateral placement of stainless steel 22 gauge guide cannulae (Plastics One, Roanoke, VA, USA) fitted with a removable dummy cannula. Coordinates were based on the atlas of Paxinos and Watson (5) and are taken from bregma (in mm) for NAc: A.P. +1.2, M.L. ±1.5, D.V. -6.50 and for BLA: A.P. -2.80, ± 4.90, D.V. -7.50. Cannulae were fixed to the skull with two anchoring screws and dental acrylic (Duralay 2244; Reliance, Worth, IL). After behavioral experiments animals were sacrificed by i.p. pentobarbital injection and correct cannulae placements was routinely verified with Evans blue histology.

Drug infusions. Behavioral experiments involving mitochondrial complex inhibitors were performed 10 min after drug administration. Behavioral experiments involving pharmacological inactivation were performed 30 min after drug administration. We randomly assigned animals to their respective treatment. For intra-cerebral infusions the dummy was removed and an injector was inserted that extended 1- (BLA) or 2mm (NAc) from the tip of the cannulae. All drugs were bilaterally infused in a total volume of 0.3 μL during 1 min of constant flow. The injector remained in place for one additional minute after infusion to allow proper diffusion. 0.224nM rotenone (complex I-inhibitor, Sigma-Aldrich) was dissolved in DMSO. 0.849nM Malonic Acid and 0.742nM 3-nitroproprionic acid (complex II-inhibitors, Sigma-Aldrich) were dissolved in saline. Muscimol (Tocris Biosciences, Bristol, UK) was dissolved in saline. All inhibitors were infused at a dose of 25ng. Mitoquinone mesylate (mitoQ) was dissolved in saline with 10% DMSO and bilaterally infused (0.5 μL) at a concentration of 10μM

3 h prior to social competition or brain sampling for the assessment of 4HNE. 57.8 nM nicotinamide (NAM, Sigma-Aldrich) was dissolved in saline and bilaterally infused at a dose of 2μg 3 h prior to social competition or respirometry. No animal received a repeated ETC drug infusion.

Auditory Fear Conditioning. Naïve rats received intra-BLA infusions of saline, 3-nitroproprionic acid, or muscimol prior to the training session of auditory fear conditioning. Animals were tested for acquisition of the fear memory by exposure to the auditory stimulus one week after training. Training and testing took place in a rodent fear conditioning cage (30×37×25 cm) placed into a sound-attenuating chamber. The side walls of the fear conditioning cage were constructed of white metacrylate, while the door and the top cover were Plexiglas. The floor consisted of 20 steel rods through which a scrambled shock from a shock generator could be delivered (Panlab, S.L., Barcelona, Spain). Following drug infusions, the animals were placed in the conditioning chambers. During the training session, rats were exposed to one context (consisting of white rectangular walls, a steel grid floor, white lights, that was cleaned with 5% ethanol before each trial) for a duration of 160s, followed by three presentations of tone-shock pairings in which the tone (20s, 80dB, 800Hz) co-terminated with a foot shock (0.6mA, 1s). The intertone interval was 40s. One week after the training session, the animals underwent a test session in a novel context (metallic grid walls, a gray plastic floor, green lights, that was cleaned with 1% acetic acid before each trial) for 8 min where the same training tone was presented during the last 5 min. Acquisition of fear was quantified as the amount of time the rats spent freezing during the testing session. Freezing was defined as the lack of all movement (except for respiratory-related movements).

Triple labeling Immunofluorescence. *Preparation.* Rats were anaesthetized with a lethal dose of pentobarbital and sacrificed by transcardial perfusion using 0.9% saline solution followed by a fixative solution of paraformaldehyde 4% in PBS (pH=7.5). The brains were removed, post-fixed for 4 h in 4% paraformaldehyde/PBS and cryoprotected in 30% sucrose/PBS. Coronal sections (30 µm thick) were cut on a cryostat (Leica, CM3050 S), and free-floating sections were triple labeled for cFOS, DAPI, and one of four antibodies against the main cell-types of the NAc. The floating sections were rinsed briefly with PBS then blocked 1 h in PBS - 0.1% Triton X-100 (Sigma-Aldrich) - 5% normal donkey serum (Jackson ImmunoResearch) and incubated overnight at 4 °C with rabbit anti-cFOS (Milllipore, ABE457, 1:500) and one of the following: goat anti-Substance P (for staining of D1-containing cells, Santa Cruz, sc-9758, 1:100), mouse anti-S-100 (for staining of astrocytes, Abcam, ab4066, 1:100), or goat anti-vesicular acetylcholine transporter (VAChT, for staining of cholinergic cells, Millipore, ABN100, 1:2,000). Rabbit anti-Met Enkephalin (for staining of D2-containing cells, Abcam, ab22620, 1:1,000) was incubated with goat anti-cFOS (Santa Cruz, sc-52-G, 1:50). Both cFOS antibodies were confirmed as exhibiting similar staining in a cFOS/cFOS double labeled control experiment. The sections were washed in PBS and incubated for 2 h at room temperature with the secondary antibodies: donkey-anti-rabbit IgG Alexa 568 conjugate (Lifetechnologies, A10042, 1:1,000), donkey anti-goat IgG Alexa 488 conjugate (Lifetechnologies, A11055, 1:800) or goat anti-mouse IgG Alexa Fluor 488 conjugate (Lifetechnologies, A11029, 1:800). After washing in PBS the sections were incubated 10 minutes in DAPI (Sigma,

1:10,000), rinsed and mounted with Fluoromount-G (SouthernBiotech). Images were captured with a confocal microscope (Zeiss, LSM700) using a × 20 objective. The sample images were captured at the same coordinates for each animal. A mosaic of 16 images were captured and stitched together for one hemisphere. For visualization purposes, representative regions within specific sections were zoomed and enhanced in a linear manner for brightness and contrast using FIJI software. Enhanced images were then arranged within photoshop CS (Adobe). Quantification was performed on original, unenhanced images only.

Quantification of immunofluorescence LSM images were stitched together using the grid stitching plug-in for FIJI (6). The backgrounds of each channel were measured at five different random areas around the section and averaged together to generate a mean background for each channel. This mean background was then subtracted from each channel. Cells were delineated using a Huang threshold to label only those stained with DAPI within 20-200 pixels. The number of these cells that were also labeled with cFOS and the antibody of interest were counted and converted to a percentage of the total number of DAPI-stained cells for each section. Sections were then averaged to provide one value per animal per cell-type of interest.

Microarray. Naïve rats were characterized for trait anxiety on the elevated plus maze (high-, intermediate-, and low- anxious rats, n=5/group) and were sacrificed under basal conditions by rapid decapitation. Brains were flash frozen and NAc were tissue-punched on a freezing cryostat with a 2.0mm tissue punch (Harris UniCore, USA). RNA was extracted using Ambion RNaqueous-micro kit (Life Technologies) which were then converted to cDNA libraries using Nugen Ovation Pico WTA system v2 reaction kits (Nugen, USA). cDNA was fragmented and labeled using the Encore Biotin Module (Nugen, USA) and hybridized to Affymetrix 1.0 Exon ST arrays (Affymetrix). The arrays were scanned to produce raw signal intensity values for all probes. These values were then preprocessed using the Robust Multichip Average algorithm (RMA; (7). Gene expression was analyzed using the *limma* package (8) within Bioconductor (9) in order to generate a model that treated anxiety as a linear predictor and produce a rank-ordered list of p-values. This rank-ordered list was then used to perform gene enrichment analysis using the GSEA software (10). We first searched for significantly associated gene sets within the C5 subclass (GO gene sets), taking a cutoff of a nominal p-value of 0.10. As the top biological processes that were differentially regulated between anxiety phenotypes were metabolic, we then examined our data for enrichment of mitochondrial genes using a custom curated gene set.

Western Blots. Rats were sacrificed under basal conditions by rapid decapitation and the nucleus accumbens was tissue-punched on a freezing cryostat with a 2.0 mm tissue punch (Harris UniCore). Briefly, tissue was homogenized in ten volumes of ice-cold sucrose (0.32 M) and HEPES (5 mM) buffer that contained a cocktail of protease inhibitors (Complete TM, Roche, UK) with 16 strokes and centrifuged at 1, 000 x g for 5 min. The resulting total fraction pellet was resuspended in Krebs buffer with 1% NP-40, incubated at 4°C for 40 min, and then centrifuged at 10,000 x g for 20 min at 4°C. Protein concentration for each sample was estimated by BCA protein analysis (Bio-Rad). 15μg of protein were loaded in each well and then separated on 10% (w／v) SDS-PAGE and transferred (70V, 1.5 h) to a nitrocellulose membrane (Whatman). Multiple gels were loaded in a counterbalanced manner and run simultaneously for

two separate experiments. After saturation of the nonspecific sites with 5% (w/v) skim milk in PBST, the blots were incubated overnight at 4C with primary antibodies against the mitochondrial complexes I-V (MitoProfile Total OXPHOS Rodent WB Antibody cocktail, MitoSciences, cat No. MS604). Polyclonal mouse anti-rat antibodies for Actin (1:30,000; Life Technologies, cat. No.9485) were incubated as loading controls. The blots were washed with PBST, incubated for 1 h with a secondary antibody, an anti-rabbit (anti-mouse for loading controls) Ig peroxidase conjugate (whole molecule conjugate; diluted 1:10,000; Sigma) and finally developed using an enhanced chemiluminescence (ECL) system (Pierce). For the quantification, bands were revealed with a ChemiDoc imaging system (Bio-Rad) for optimum exposure time. Images were then analysed using QuantityOne software v4.6.3 (Bio-Rad) where the adjusted volume was calculated and recorded for each band. For each group, adjusted values were normalized to Actin.

Mitochondrial Copy Number. Rats were sacrificed under basal conditions by rapid decapitation and the nucleus accumbens was rapidly dissected out, frozen at -30°C in isopentane, and stored at -80°C until further processing. Tissue was placed in lysis buffer and DNA was extracted using DNeasy extraction kit (Qiagen). DNA concentrations were measured using the Nanodrop. Oligonucleotide primers for real-time quantitative PCR and tested to ensure all efficiencies were above 95%. All primers were efficient between 97.8 and 99.7%. Primers tested includ: ND1, fwd TCCTCTTATCCGTCCTCCTAATAA; rev CAGGCGGGGATTAATAGTCA; GAPDH, fwd AAACCCATCACCATCTTCCA; rv CCTCGAAGTACCCTGTGCAT. Each reaction contained 200 nM each of the forward and reverse primers, SYBR Green PCR Master Mix (Applied Biosystems) and 10 ng sample DNA in a 20 µl reaction volume. The qPCR reactions were performed in triplicates in an ABI Prism 7900 Sequence Detection System (Applied Biosystems). The standard cycling conditions were 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, and 60°C for 30 s. Melt curves were generated at the end of the regular qPCR cycles.The comparative Ct method was used to determine raw copy number. MtDNA levels were normalized to the nuclear-encoded gene, GAPDH.

Electron Microscopy. *Preparation.* Rats were anaesthetized with a lethal dose of pentobarbital and then sacrificed under basal conditions by transcardial perfusion of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M PB (pH7.4). The brains removed 2h after perfusion. Sections (80 µm) were cut in the coronal plane at the level of the NAc. Sections where washed in cacodylate buffer (0.1M, pH 7.4) and postfixed in 1% osmium tetroxide/1.5% potassium ferrocyanide, 1% osmium tetroxide, and 1% uranyl acetate, each fixation step lasting 40 min. The sections were dehydrated in increasing ethanol concentrations, before being washed in propylene oxide, and infiltrated with Epon resin. Sections where then flat embedded between two glass slides coated in mold releasing agent (Hobby time, Glorex, Switzerland) and polymerized at 60°C for 24h. Sections (50nm) where then cut through the NAc using a diamond knife and collected onto copper slot grids with a pioloform support film.

Imaging. Sections were imaged with a transmission electron microscope (Tecnai Spirit, FEI company, Eindhoven, The Netherlands) at 80 KeV and a magnification of 2.29nm x 2.29nm per pixel and images captured with a CCD camera (4k x 4k Eagle camera, FEI company).

Quantification. Mitochondrial density and size were measured blind using the TrakEM2 software in Fiji (11). A counting square, with an area of 66.9 μm^2, was drawn on each image. Mitochondria inside this square, as well as those touching the top and right edges (inclusion lines), were counted. Those touching the bottom and left were excluded (exclusion lines). The largest diameter was measured, and mitochondria were also classified as to whether they were seen in dendrites, or elsewhere. A total of 377 counting squares were analyzed from 6 animals (n = 3/group for high- and low-anxious rats).

Mitochondria Respirometry. Animals were characterized for anxiety and sacrificed under basal conditions by rapid decapitation and the nucleus accumbens and basolateral amygdala were rapidly dissected out, weighed, and placed in a petri dish on ice with 2 mL of relaxing solution (2.8 mM Ca_2K_2EGTA, 7.2 mM K_2EGTA, 5.8 mM ATP, 6.6 mM $MgCl_2$, 20 mM taurine, 15 mM sodium phosphocreatine, 20 mM imidazole, 0.5 mM dithiothreitol and 50 mM MES, pH = 7.1) until further processing. Tissue samples were then gently homogenized in ice-cold respirometry medium (MiR05: 0.5 mM EGTA, 3mM $MgCl_2$, 60 mM potassium lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose and 0.1% (w/v) BSA, pH=7.1) with an eppendorf pestle. Then, 2 mg of tissue were used to measure mitochondrial respiration rates at 37°C using high resolution respirometry (Oroboros Oxygraph 2K, Oroboros Instruments, Innsbruck ,Austria), as previously described for other tissues (12). A multisubstrate protocol was used to sequentially explore the various components of mitochondrial respiratory capacity. To measure the respiration due to oxidative phosphorylation, we added substrates for the activation of specific complexes. Thus, oxygen flux due to complex I activity (Complex I) was quantified by the addition of ADP (5 mM) to a mixture of malate (2mM), pyruvate (10mM) and glutamate (20mM), followed by the addition of succinate (10 mM) to subsequently stimulate complex II (Complex I + II). We then uncoupled respiration to examine the maximal capacity of the electron transport system (ETS) using the protonophore, carbonylcyanide 4 (trifluoromethoxy) phenylhydrazone (FCCP; successive titrations of 0.2 µM until maximal respiration rates were reached). We then examined consumption in the uncoupled state due solely to the activity of complex II by inhibiting complex I with the addition of rotenone (0.1µM; ETS CII). Finally, electron transport through complex III was inhibited by adding antimycin (2 µM) to obtain the level of residual oxygen consumption (ROX) due to oxidating side reactions outside of mitochondrial respiration. The O_2 flux obtained in each step of the protocol was normalized by the wet weight of the tissue sample used for the analysis and corrected for ROX. All respiration experiments comprise 2-3 counterbalanced blocks across days.

Quantification of ATP. Animals were characterized for anxiety and sacrificed under basal conditions by rapid decapitation. The NAc was dissected out and placed in 2 mL of relaxing solution (2.8 mM Ca_2K_2EGTA, 7.2 mM K_2EGTA, 5.8 mM ATP, 6.6 mM $MgCl_2$, 20 mM taurine, 15 mM sodium phosphocreatine, 20 mM imidazole, 0.5 mM dithiothreitol and 50 mM MES, pH = 7.1) until further processing. Tissue samples were then diluted 10x in a tricine buffer solution (40 mM Tricine, 3 mM EDTA, 85 mM NaCl, 3.6 mM KCl, 100 mM NaF and 0.1% saponin, pH 7.4; Sigma Aldrich). ATP content was determined enzymatically with luciferase in a 96-well plate. In the presence of ATP, Mg^{2+} and oxygen, luciferin is oxygenated by luciferase into oxyluciferin. This

reaction emits light which is proportional to the amount of ATP in the sample. ATP was measured with the CellTiter-Glo Luminescent Cell Viability Assay (Promega), with a few minor modifications. A converting solution (100 mM Tricine, 100 mM MgSO4, 25 mM KCl) was added to tissue samples and allowed to incubate at room temperature for 5 min. After incubation, a MgCl2 solution (4 mM tricine and 100 mM MgCl2) was added to the samples, followed by, 100 µl of CellTiter-Glo reagent (G7571, Promega). Additionally, each 96-well plate contained a series of 10-fold dilutions (1µM – 10nM) of an ATP standard (Sigma), in order to generate a standard curve for each assay. Luminescence was immediately detected with a luminometer (Safire 2, Tecan). Luminescence was measured kinetically via 30 samples taken at 1 min intervals. At least 4 points at the plateau were taken to generate an average maximum luminescence for each sample. ATP was calculated using the standard curve to determine the concentration of ATP.

Quantification of lipid peroxidation product 4-HNE. Animals were characterized for anxiety and either sacrificed under basal conditions by rapid decapitation or infused with 10µM mitoQ in the NAc and sacrificed 3 h afterwards. The brain was extracted, frozen at -30°C in isopentane and stored at -80°C until further processing. In basal samples, NAc punches were obtained using on a freezing cryostat and then processed for 4-HNE accumulation using a 4-HNE ELISA assay (Cell BioLabs, Cat.No. STA-334 and STA-838) according to manufacturer's instructions. Samples receiving mitoQ were rapidly fresh-dissected for the NAc and then processed for 4-HNE accumulation as described above.

Isolation of synaptoneurosomal- and glial-enriched fractions. To separate synaptoneurosomes from glial cells, we followed procedures for separation via percoll gradient detailed in (13). Briefly, rats were sacrificed under basal conditions by rapid decapitation and nucleus accumbens from 3 animals per group per experiment were pooled together, rapidly dissected out, and immediately homogenized in a glass tissue grinder in homogenization buffer (1.28M sucrose, 4mM EDTA, 20mM Tris, 50mM DTT, pH 7.4) by 10 even strokes. The homogenate was centrifuged at 1,000*g* for 10 min at 4°C. The supernatant was collected and carefully loaded onto a prepared percoll gradient column (consisting of layers 3, 10, 15, and 23% percoll) and then centrifuged at 31,000*g* for 5 min. at speed at 4°C. Individual synaptoneurosomal- and glial-enriched fractions were then carefully collected from each corresponding percoll layer and protein concentration was determined using the Bradford assay. Both fractions were then washed with ice-cold sucrose/EDTA buffer and centrifuged at 15,000*g* for 15 min. As these two fractions were separated for their subsequent inclusion in respiration analyses, the obtained pellets were resuspended in Mir05 solution and immediately placed inside the Oroboros chambers for respirometry measurements. Five µg of each fraction were reserved and directly loaded onto a 12.5% (w/v) SDS-PAGE for western blot analysis against mouse anti-NeuN (a neuronal marker; Millipore MAB377, 1:1,000), mouse anti-S100 (astrocytic marker; Abcam, ab4066, 1:100), and mouse anti-GAPDH (loading control, Abcam, ab8245, 1:30,000) in order to ensure successful cell separation (see methods Western Blots).

Histology. Animals were bilaterally implanted with cannulae aimed at the NAc, as described above. They received one unilateral infusion (25 ng/ 0.3 µl) of either rotenone, malonic acid, or 3-nitropropionic acid (3NP), with the opposing side receiving the corresponding vehicle (DMSO for rotenone and saline for malonic acid and 3NP treatments). Animals were sacrificed at 24h by pentobarbital injection followed by transcardial perfusion of 0.9% saline solution and paraformaldehyde 4% in PBS (pH=7.5).

H-E staining For hemotoxylin-eosin (HE) staining, brains were dehydrated using increasing steps of EtOH and embedded in paraffin. Sections were cut (10 µm), fixed in acetone for 10 min, and rinsed in distilled water. Sections were incubated with hemotoxylin for 5 min, gently rinsed in tap water for 10 min, and stained in eosin for 2 min. Sections were rinsed in distilled water, dehydrated, and mounted. Images were captured using a brightfield slide scanner (Olympus Slide Scanner VS120-L100).

Cleaved caspase 3 For cleaved caspase 3 analysis, brains were removed, post-fixed for 4 h in 4% paraformaldehyde/PBS and cryoprotected in 30% sucrose/PBS. Coronal sections (30 µm thick) were cut, and free-floating sections were then labeled with a primary antibody against cleaved-caspase 3. Briefly, the floating sections were rinsed with Tris-buffered saline/0.1% Triton-X-100 (TBS-T), and endogenous peroxidases were blocked by incubation in 0.3% H2O2/TBS-T. After washing, sections were blocked in 5% normal donkey serum/TBS-T and incubated overnight at 4°C with antibodies against rabbit anti-cleaved caspase 3 (Cell Signaling, 9661s, 1:400). The sections were washed in TBS-T and incubated for 1 h at room temperature with biotinylated goat anti-rabbit IgG (both 1:200; Vector Laboratories). Images were scanned using a brightfield slide scanner at 20x magnification (Olympus Slide Scanner VS120-L100) and converted to tiff images using the VSI reader actionbar for ImageJ. Images were adsorbed, regions of interest at the site of drug infusion were drawn, and light intensity was measured. At least two sections from each animal were measured and averaged to generate one value per hemisphere per animal for each drug infusion.

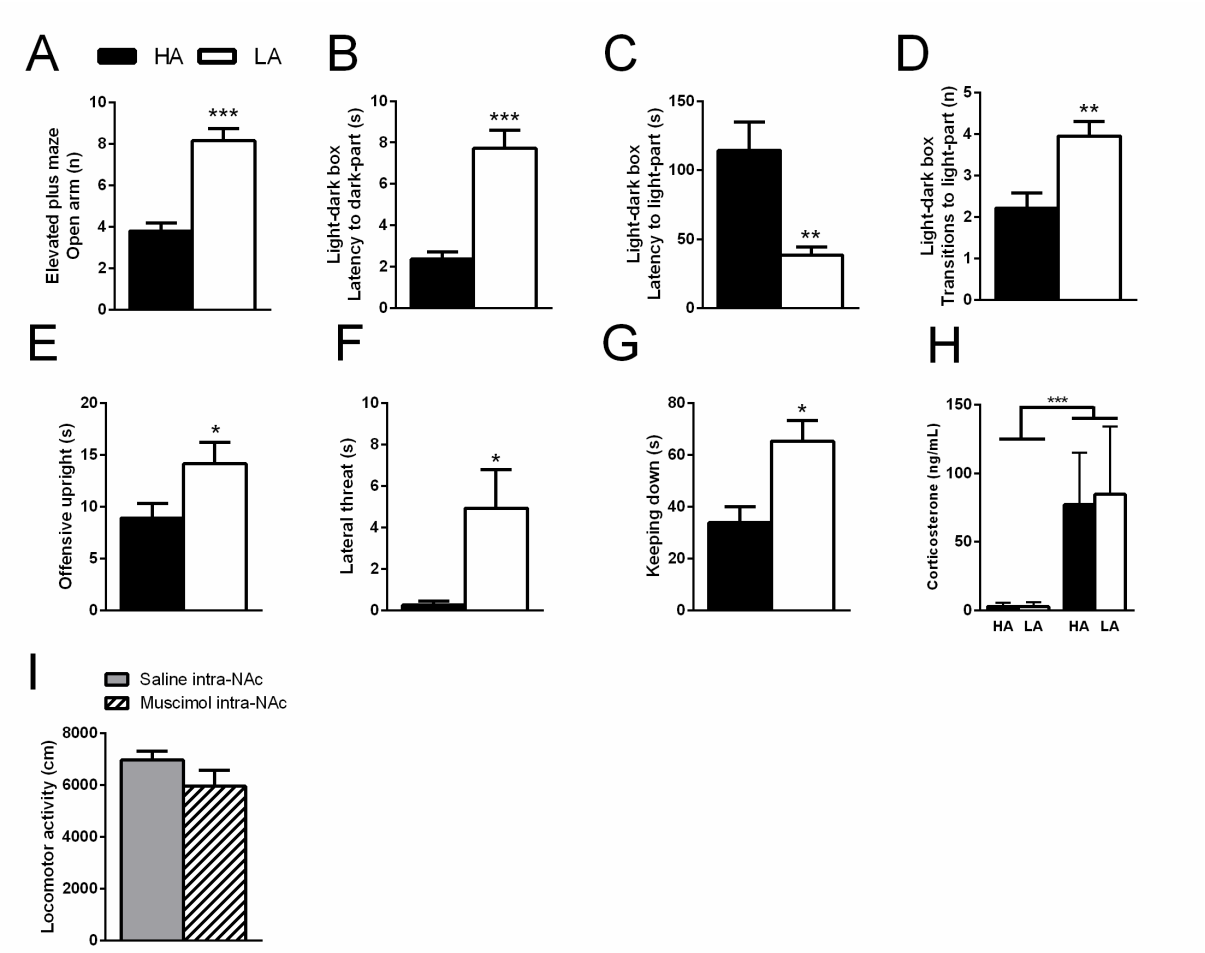

Fig. S1. Characterization of high-anxious and low-anxious rats**.** Rats exhibiting a high- (HA) or low-anxiety (LA) profile based on time spent on the open arms of the elevated plus maze (EPM) also differed in the number of open arm entries on the EPM (**A**). In the light-dark box, high-anxious rats differed from low-anxious rats on all relevant parameters in the expected direction; a reduced latency to enter the lit compartment (**B**), an increased latency to enter the lit compartment (**C**) and reduced number of total dark-light transitions (**D**), *n*= 22-24/group. During social competition, HA rats displayed a reduced duration of behaviors associated with social dominance: offensive upright (**E**), lateral threat (**F**) and keeping down (**G**), *n*= 24/group. Following social competition, corticosterone levels were significantly increased compared to home cage controls, though at both time points, HA and LA rats exhibit similar corticosterone levels (**H**), *n*= 10-20/group. Intra-NAc muscimol did not affect locomotor activity in the open field (**I**), *n*= 8/group. Data are presented as mean ± SEM (*p<0.05, **p<0.01; ***p<0.001, Student's t-test).

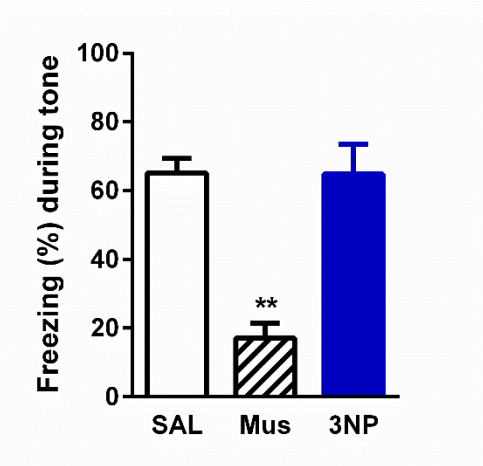

Fig. S2. Acoustic fear conditioning is unaffected by the infusion of the complex II non-competitive inhibitor 3-nitroproprionic acid (3NP) into the basolateral amygdala (BLA). Whereas, intra-BLA infusion of muscimol (Mus; *n*= 4) before training in the acoustic fear conditioning task markedly reduced freezing in response to a tone during the testing session, administration of 3NP (*n*= 8) had no effect on freezing at testing, as compared to saline (SAL; *n*= 5) controls. Data are presented as mean ± SEM (**p<0.01; one-way ANOVA, bonferroni post-hoc comparisons).

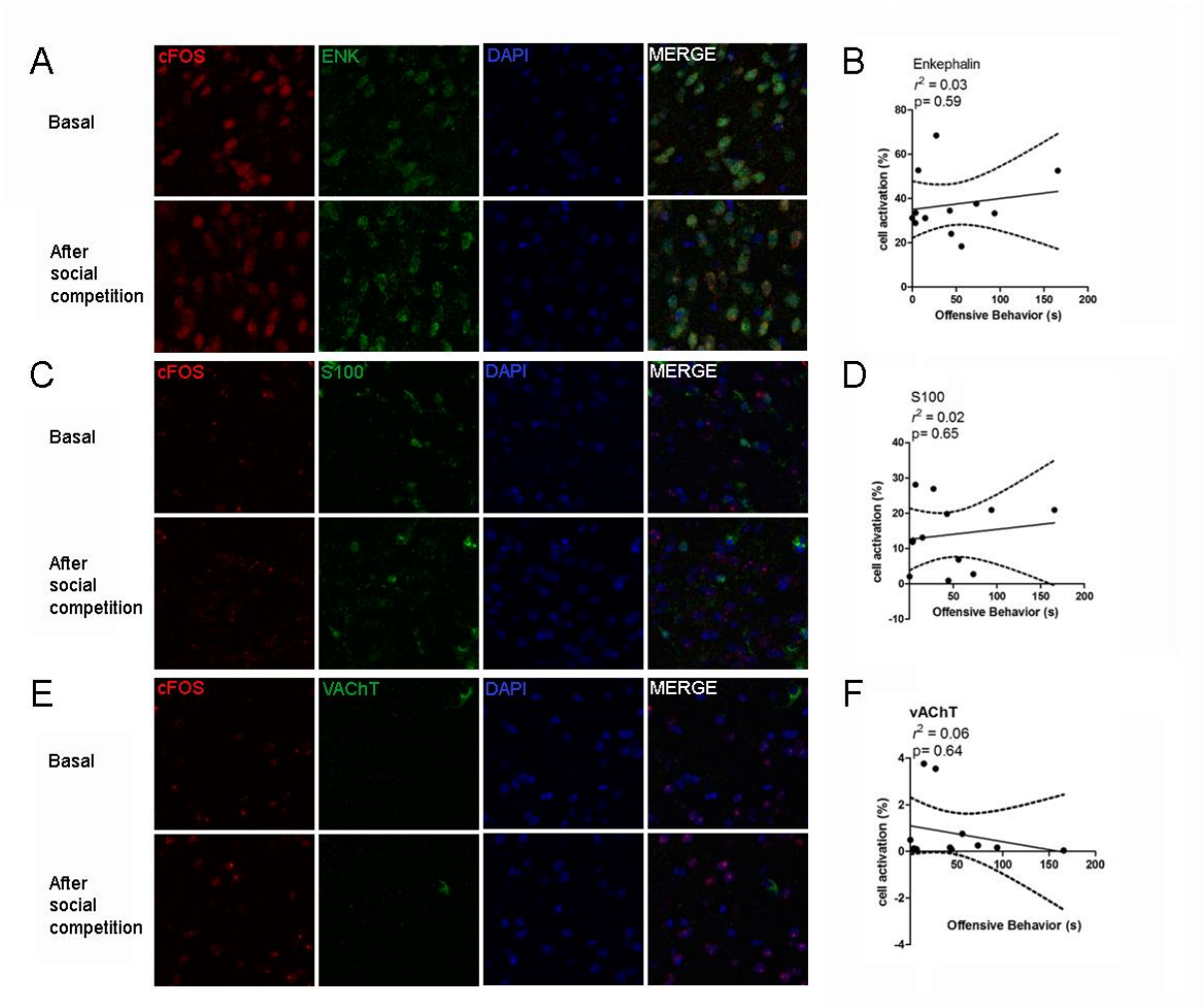

Fig.S3. Representative images of enkephalin (ENK, for D2-containing cells; **A**), S100 (for astrocytic cells; **C**), and vesicular acetylcholine transporter (VAChT, for cholinergic cells; **E**) under basal conditions and following social competition. Double-labeling with cFOS found that neither ENK, S100, nor VAChT correlate significantly with the amount of offensive behavior (**B, D, F**).

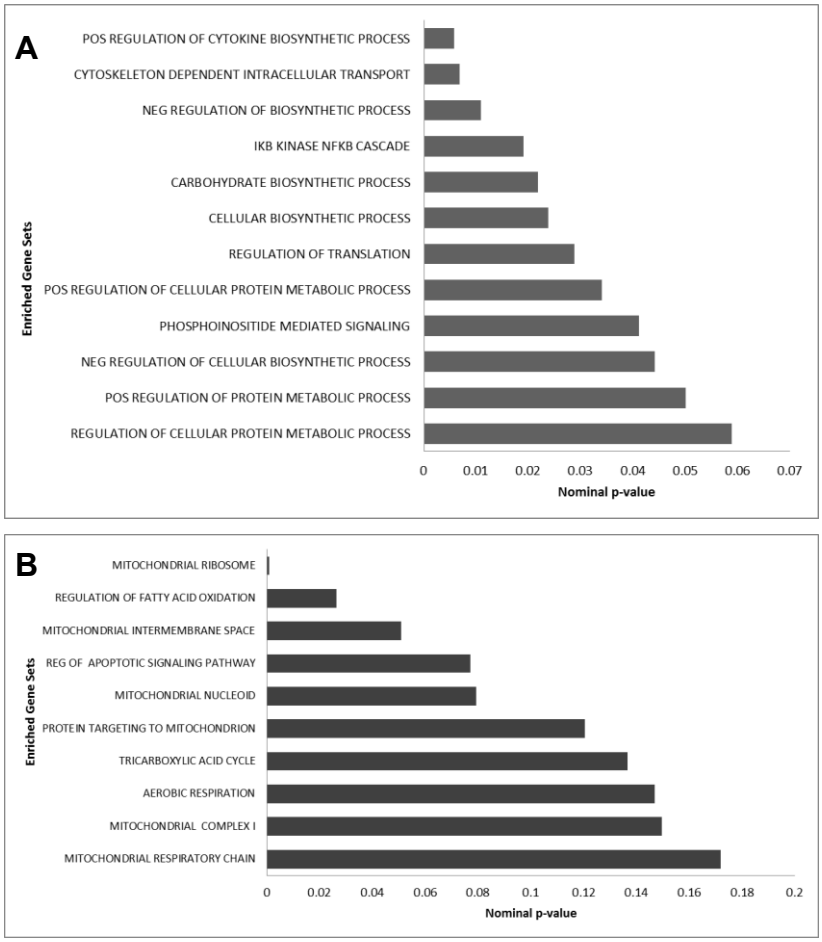

Fig. S4. Gene enrichment analysis indicates a pattern of differential gene expression for metabolic processes and functions within the NAc between high- and low-anxious rats. Gene expression microarrays on high- and low-anxious rats under basal conditions (*n*= 5/group) were performed and analyzed for enrichment using Gene Set Enrichment Analysis (GSEA) software when examining across general GO gene sets (**A**) Enrichment analysis on a curated set of mitochondrial genes identified significantly enriched gene sets related to mitochondrial organization and function (**B**).

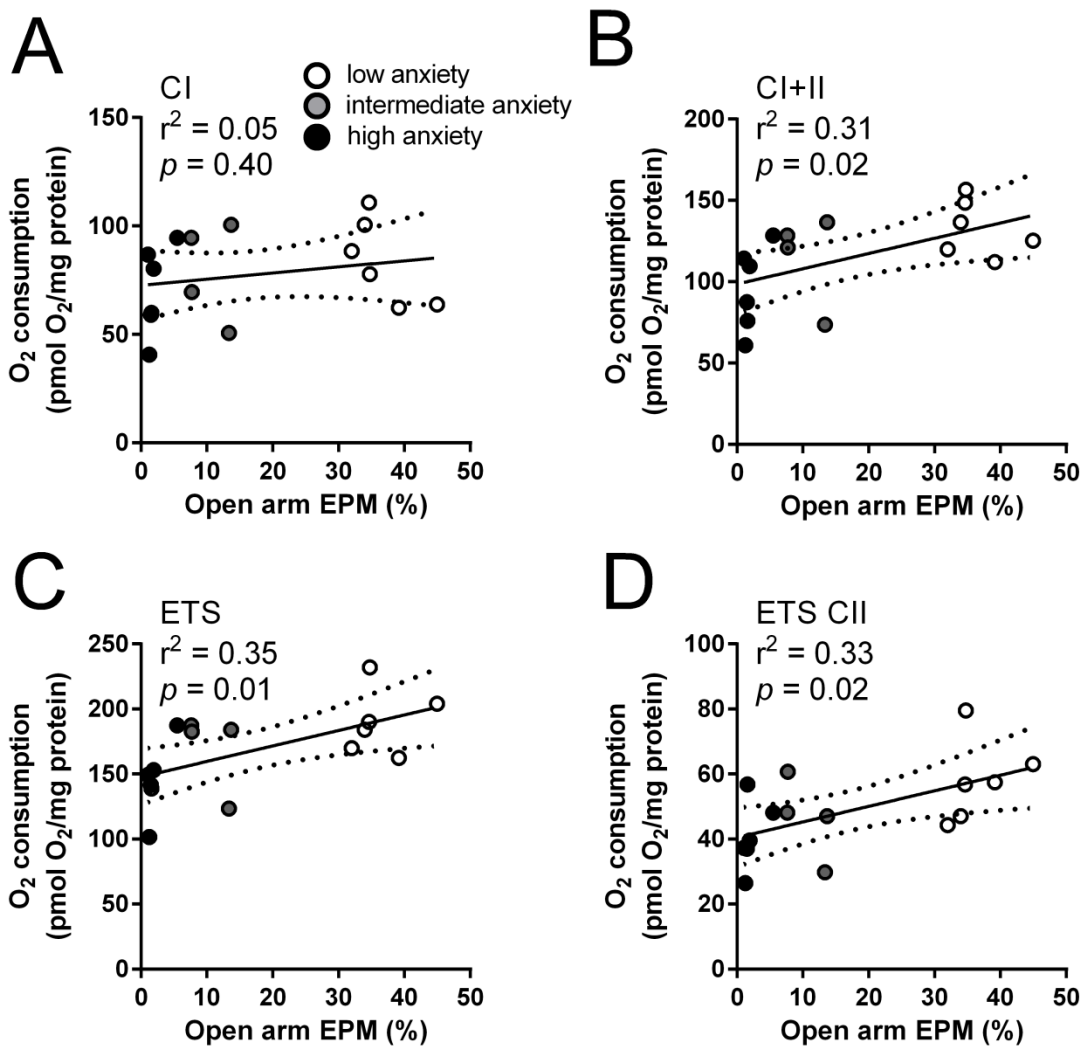

Fig. S5. Correlations between anxiety-like behavior on the elevated plus maze and oxygen consumption for (**A**) stimulation of complex I (CI) by addition of ADP; (**B**) stimulation of complex II (CII) by addition of succinate; (**C**) maximal electron transport system capacity (ETS) by addition of FCCP; and (**D**) maximal capacity due only to complex II (ETS CII) by addition of rotenone. Coefficient of determination (r^2) and p-values calculated from linear regressions.

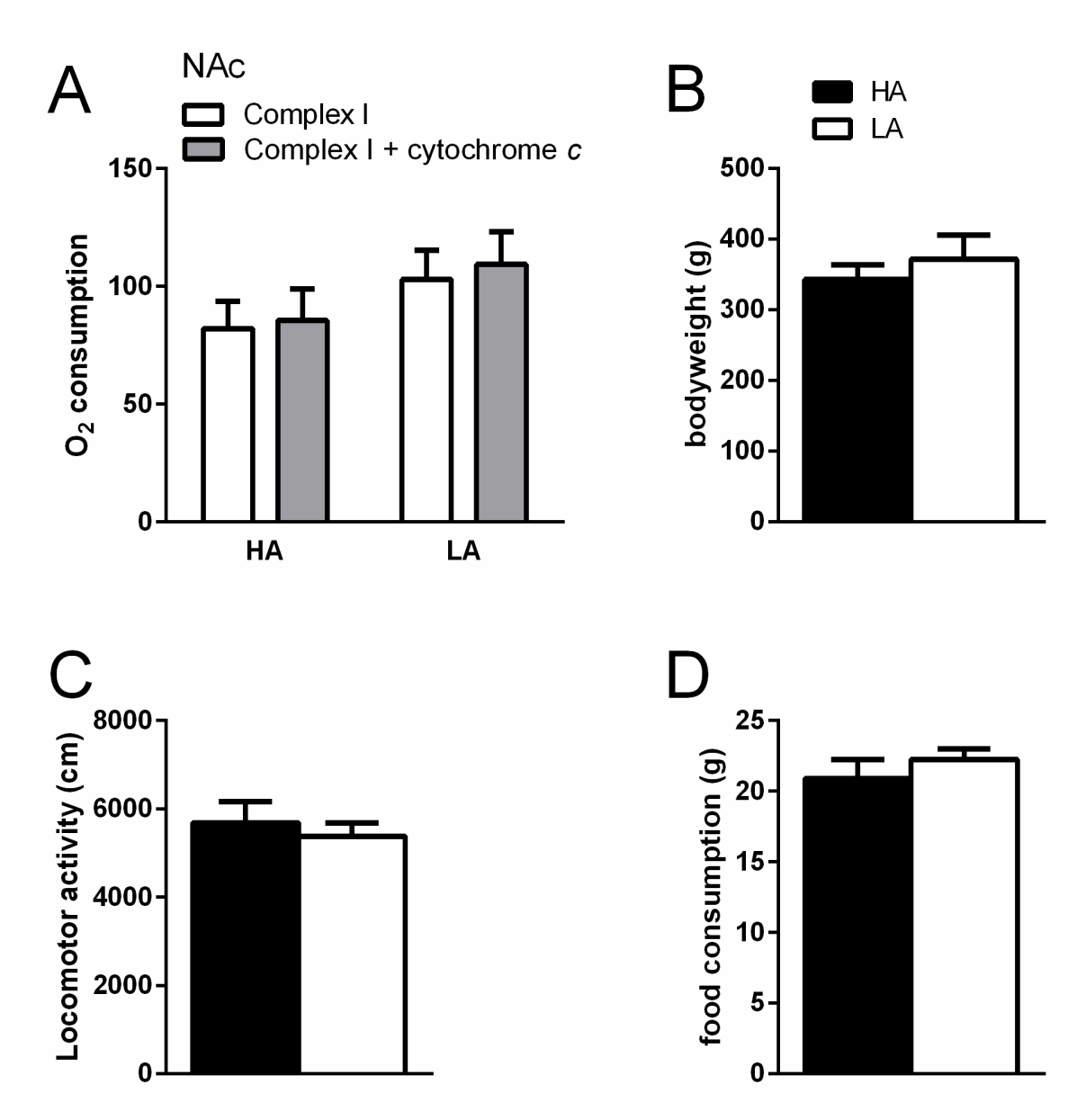

Fig. S6. The difference in mitochondrial respiration between high-anxious (HA) and low-anxious (LA) rats was not the result of differential mitochondrial vulnerability. When mitochondria are damaged, the addition of cytochrome c will replace what had leaked out and therefore enhance respiration. Here, we show that both HA and LA homogenates are unaffected by the addition of 10µM cytochrome c, indicating no differential damage upon processing (**A**). Data are presented as estimated marginal means ± SEM of oxygen flux per mg tissue using a two-way repeated-measures Linear Mixed Model, n=8/group. Differences in trait anxiety respiration were not related to differences in adult body weight (**B**), locomotor activity in the open field (**C**) or overnight food intake (**D**), *n*= 7-11/group.

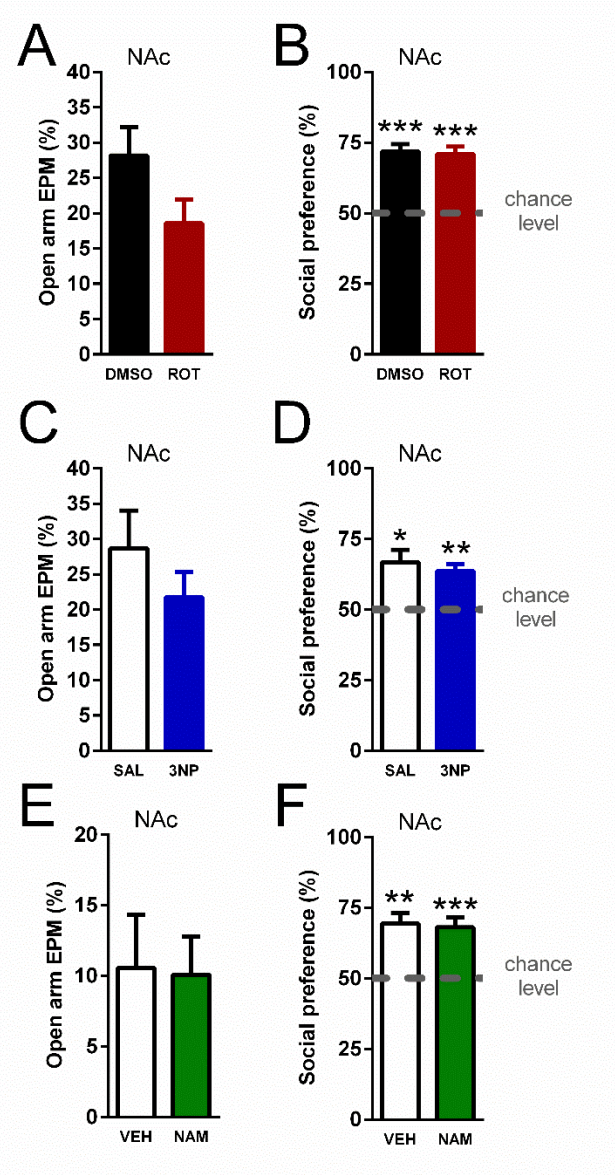

Fig. S7. No effect of mitochondrial treatment on anxiety and social preference. Intra-NAc infusion of rotenone (ROT) had no effect on anxiety (**A**) or social preference (**B**). Infusion of 3-nitroproprionic acid (3NP) similarly did not affect anxiety (**C**) or social preference (**D**). Intra-NAc infusion of nicotinamide (NAM) also did not affect anxiety (**E**) or social preference (**F**), *n*= 9-12/group. Data are presented as mean ± SEM (**p<0.01, ***p<0.001, Student's t-test or one-sample t-test against chance- level).

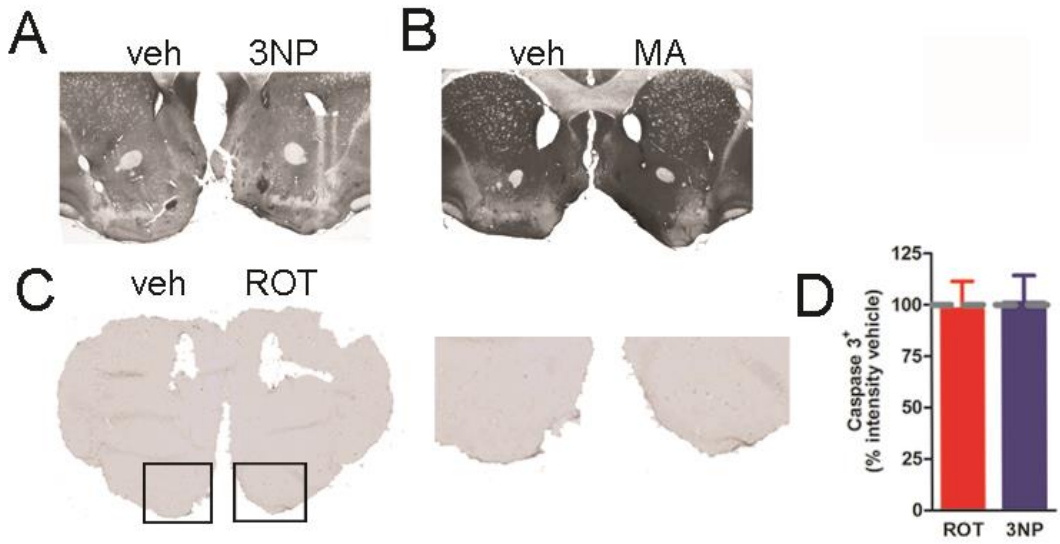

Fig. S8. The intra-NAc infusion of malonic acid (MA) or 3-nitroproprionic acid (3NP) at the dosing scheme used in the current study (25ng/hemisphere, 10 min prior to behavioral testing) did not affect gross brain morphology as shown by HE-staining (**A,B**). Nor were there effects of the mitochondrial inhibitors on apoptotic cell death (**C-D**) as evidenced by the amount of cleaved-caspase 3. Data (**D**) are represented as mean ± SEM (n= 3/group, Student's t-test).

Supplementary Table 1: Differentially expressed genes between high- and low-anxious rats from curated mitochondrial gene sets

mRNA Accession #	Gene Symbol	Gene Description	GO Accession	GO name	P-Value
NM_001002253	Atp6v0e2	ATPase, H+ transporting V0 subunit e2	> GO:0015991	ATP hydrolysis coupled proton transport	0.005
NM_053994	Pdha2	Pyruvate dehydrogenase (lipoamide) alpha 2	> GO:0004739	Pyruvate dehydrogenase activity	0.006
NM_001108898	Ddx28	DEAD (Asp-Glu-Ala-Asp) box polypeptide 28	> GO:0042645	Mitochondrion nucleoid	0.009
NM_001013431	Chchd4	Coiled-coil-helix-coiled-coil-helix domain containing 4	> GO:0006626	Protein targeting to mitochondria	0.012
NM_053970	Nln	Neurolysin (metallopeptidase M3 family)	> GO:0005739	Mitochondrion	0.031
NM_017254	Htr2a	5-hydroxytryptamine (serotonin) receptor 2A	> GO:0006816	Calcium ion transport	0.031

Table S1. Differentially expressed genes between high- and low-anxious rats identified in the custom-curated mitochondrial gene sets following gene enrichment analysis. P-values were obtained from a general linear model of anxiety and gene expression.

Supplementary Table 2: Mitochondrial density and length in high- and low-anxious rats

Measurement	HA	LA	p-value
Total Density (per μm^2)	0.75±0.05	0.70±0.06	0.53
Density within dendrites (per μm^2)	0.50±0.04	0.50±0.05	0.98
Total Length (μm)	438.3± 17.3	423.5± 25.7	0.66

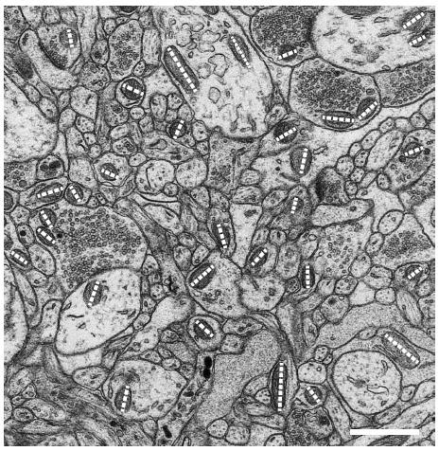

Table S2. Total mitochondrial density, density within dendrites and total mitochondrial length in the nucleus accumbens measured in images captured by electron microscopy did not differ between high- (HA) and low-anxious (LA) rats. Density is presented as mean mitochondria per μm^2 ± SEM (Student's t-test). Length is presented as mean µm per 1500 mitochondria ± SEM (Student's t-test). In the lower panel, a TEM representative image highlighting mitochondria with internal inserts is shown.

Supplementary Table 3: Effects of anxiety and NAc treatments on other social behaviors

Dyad Composition	Condition	Social Investigation (s)	p-value	Auto-grooming (s)	p-value
HA	Basal	128.3 ± 10.0	0.94	41.1 ± 6.7	0.57
LA	Basal	129.5 ± 12.5		36.2 ± 5.9	
Matched	ROT	156.9 ± 23.0	0.36	87.9 ± 20.6	0.39
	DMSO	129.2 ± 24.2		56.7 ± 20.2	
Matched	MA	122.1 ± 16.5	0.83	46.5 ± 10.6	0.49
	Saline	134.8 ± 18.5		42.8 ± 9.6	
Matched	3NP	161.4 ± 23.4	0.57	51.0 ± 9.6	0.91
	Saline	164.6 ± 21.3		64.8 ± 13.7	
HA	NAM	143.5 ± 29.0	0.47	72.8 ± 17.4	0.89
HA	Saline	118.8 ± 16.4		76.2 ± 23.2	
Matched	MUS	107.5 ± 27.5	0.02	43.7 ± 15.5	0.89
	Saline	211.1 ± 27.5		40.3 ± 16.9	

Table S3. The specificity for each of the pharmacological treatments given intra-NAc on social competition was investigated by measuring the total duration of social investigation and auto-grooming during the social encounter. Dyads consisted of either high- and low-anxious (HA, LA respectively) rats or anxiety-matched rats (Matched). Abbreviations: ROT, rotenone; DMSO, dimethylsulfoxide; 3NP, 3-nitropropionic acid; MA, malonic acid; NAM, nicotinamide; MUS, muscimol. Data are presented as mean ± SEM (Student's t-test).

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